UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

March 9, 2007
TXR # 0054533

SUBJECT THIAMETHOXAM. Review of Developmental Neurotoxicity Study including Brain Morphometry Data in Low- and Mid-Dose Groups.

PC Code: 060109
DP Barcode: D332732

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I. CONCLUSIONS

Attached is the second review for a developmental neurotoxicity study in rats with Thiamethoxam (MRID 46028202, main study, and MRID 46028201, preliminary study). This review includes additional data recently submitted for brain morphometric measurements in low- and mid-dose groups (MRID 47034201).

The maternal NOAEL was 400 ppm (34.5 mg/kg/day) and the maternal LOAEL was 4000 ppm (298.7 mg/kg/day) based on decreases in body weight gain and food consumption.

The offspring NOAEL was 400 ppm (34.5 mg/kg/day) and the offspring LOAEL was 4000 ppm (298.7 mg/kg/day) based on decreased body weight and body weight gain in males and females, delayed sexual maturation in males, and reduced brain weight and size in males and females.

This study is classified **Acceptable/Non Guideline** and may be used for regulatory purposes. **It does not, however, satisfy the guideline requirement for a developmental**

neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft) pending comprehensive review of the positive control data.

II. BACKGROUND

The original developmental neurotoxicity (DNT) study report only had brain morphometric measurements in low- and mid-dose groups if changes in the high-dose group were statistically significant at the 0.01 level. The DNT Workgroup requested that brain morphometric measurements also be made in low- and mid-dose groups when changes in the high-dose group were significant at the 0.05 level and in sections contiguous to sections with substantial changes (March 6, 2007 memo, TXR # 0054519).

This submission (MRID 47034201) includes the requested morphometric measurements and updates the original DER (MRID 46028202, TXR # 0052145).

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TXR#: 0054533

DATA EVALUATION RECORD

STUDY TYPE: Developmental Neurotoxicity Study - Rat; OPPTS 870.6300 (§83-6); OECD 426

PC CODE: 060109 **DP BARCODE**: D332732

TEST MATERIAL (PURITY): Technical Grade Thiamethoxam (98.8%)

SYNONYMS: CGA 293343

CITATION: Brammer, A. (2003) Thiamethoxam: Developmental Neurotoxicity Study in Rats. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Study number RR0936; May 29, 2003. MRID 46028202. Unpublished

Brammer, A. (2003) Thiamethoxam: Preliminary Developmental Neurotoxicity Study in Rats. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Study number RR0935; May 22, 2003. MRID 46028201. Unpublished

Brammer, A. (2007) Thiamethoxam: Supplement to Developmental Neurotoxicity Study in Rats: (Supplemental to MRID Number 46028202). *Project Number*: RR0936/REG/S1, RR1101, T011575/06. Central Toxicology Lab. (Syngenta). MRID 47034201

SPONSOR: Syngenta Crop Protection, Inc., 410 Swing Road, Post Office Box 18300, Greensboro, NC 27419.

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (MRID 46028202), Thiamethoxam (98.8% a.i., batch # P 506006) was administered to 30 pre-mated female Alpk:AP$_f$SD (Wistar-derived) rats/dose in the diet at concentrations of 0, 50, 400 or 4000 ppm from gestation day (GD) 7 through postnatal day (PND) 22. The average daily intake of Thiamethoxam during gestation was 0, 4.3, 34.5 and 298.7 mg/kg/day, respectively and 0, 8.0, 64.0 and 593.5 mg/kg/day, respectively, during lactation. Dose levels were chosen based on the results from a preliminary developmental neurotoxicity study in the rat (MRID 46028201). Additional brain morphometric measurements were later conducted on low- and mid-dose groups (MRID 47034201).

A Functional Operational Battery (FOB) was performed on 30 dams/dose on gestation days 10 and 17 and on lactation days 2 and 9. On PND 5, litters were culled to yield four males and four

females (as closely as possible). Offspring representing at least 20 litters/dose were allocated for FOB observations (FOB) and assessments of motor activity, auditory startle response, learning and memory and neuropathology at study termination (day 63 of age). The age of developmental landmarks, vaginal opening in females and preputial separation in males, was recorded. On PND 12, the whole brain was collected from at least 10 pups/sex/dietary level for weight; histopathological and morphometric examinations were conducted on control and high dose animals. On PND 63, the brains of at least 10 pups/sex/dietary level were weighed. Also on day 63, another 10 rats/sex/group were sacrificed for brain histopathology and morphometrics; only the control and high dose groups were examined, except for areas of the dorsal cortex, thalamus and hippocampus, which were evaluated in the low and intermediate groups.

There were no maternal deaths or treatment-related findings during the general observations and the FOB battery tests. The following were observed in females at 4000 ppm: significantly decreased (95-96% of control value) body weight during gestation; decreased (88% of control value) body weight gain during gestation; significantly decreased (80-83% of control value) food consumption during the latter part of gestation (days 7-15 and 15-22); significantly decreased (93-97% of control value) body weight beginning on LD 1 and continuing to LD 22; and significantly decreased (80-91% of control value) food consumption throughout lactation. No treatment-related findings were observed in females at 50 or 400 ppm.

The maternal toxicity LOAEL was 4000 ppm (298.7 mg/kg/day) based on decreases in body weight gain and food consumption. The maternal NOAEL was 400 ppm (34.5 mg/kg/day).

Treatment had no adverse effects on offspring survival, clinical signs, FOB, motor activity, auditory startle reflex, learning and memory, or neuropathology.

No treatment-related effects were seen on body weight, body weight gain, food consumption, brain weights or brain morphology at the low and the mid dose groups. At the high dose (4000 ppm), body weight of male and female pups were decreased on Days 1 (91-93% of control value) and Day 5 (pre-cull) (95-96% of control value) which remained lower (88-96% of control value) throughout lactation. Body weight gain were decreased (80-87% of control value) during lactation. Also at the high dose, the mean age for preputial separation was significantly delayed in male pups. Absolute brain weight was statistically significantly lower in males and females at 4000 ppm on days 12 and 63. On day 12, the length and width of the cerebellum was significantly lower in males at 4000 ppm. On day 63, significant decreases in Level 3-5 measurements were observed in males and in Level 4-5 in females at 4000 ppm.

The offspring LOAEL was 4000 ppm (298.7 mg/kg/day) based on decreased body weight and body weight gain in males and females, delayed sexual maturation in males, and reduced brain weight and size in males and females. The offspring NOAEL was 400 ppm (34.5 mg/kg/day).

This study is classified **Acceptable/Non Guideline** and may be used for regulatory purposes. **It does not, however, satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft) pending comprehensive review of the positive control data.**

COMPLIANCE: Signed and dated Flagging, GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:** Technical grade Thiamethoxam

Description:	Beige/yellow solid
Batch #:	P 506006
Purity:	98.8 % a.i.
Compound Stability:	Stable in the diet for up to 57 days at room temperature
CAS # of TGAI:	153719-23-4

2. **Vehicle and/or positive control:** none

3. **Test animals (P):**

Species:	Rat	
Strain:	Wistar-derived (Alpk:AP$_f$SD)	
Age at study initiation:	Time-mated females: 10-12 wks	
Wt. at study initiation:	218-313 g	
Source:	Rodent Breeding Unit (RBU), Alderly Park, Macclesfield, Cheshire	
Housing:	Individually in solid plastic cages	
Diet:	CT1 diet (Special Diets Services, Limited, Witham, Essex, UK), *ad libitum*	
Water:	Tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	22±3 °C
	Humidity:	30-70%
	Air changes:	At least15/hour
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	Six days	

B. PROCEDURES AND STUDY DESIGN:

1. **In life dates**: Start: March 25, 2002; End: February 13, 2003

2. **Study schedule:** Time-mated female Alpk:AP$_f$SD rats (30/dose group) were administered the test material in the diet from gestation day 7 through parturition and lactation to day 22 *post partum*. On day 5 *post partum*, litters were standardized to 8 pups, sexes were represented as equally as possible. Pups were weaned from the dam on day 29; dams were sacrificed after the weaning. Pups remained on study up to PND 63.

3. **Mating procedure:** Time mating of females was carried out at Rodent Breeding Unit. The day that sperm was detected in a vaginal smear was designated gestation day (GD) 1. Females with sperm positive smears were delivered to the testing laboratory on GD 1; 16 females were supplied on each of 7 days and 8 females on the eighth day.

4. **Animal assignment:** The study was conducted using a replicate (randomized block) design. The mated females were randomly assigned to treatment groups upon arrival at the testing laboratory, as shown in Table 1. On gestation days 10 and 17 and lactation days (LD) 2 and 9, the females were examined outside the cage using a functional observation battery (FOB) of tests.

   At least 10 pups/sex/group (one male or one female from each litter) were observed on PNDs 5, 12, 22, 36, 46 and 61. One male or one female from each litter was assessed for motor activity on PNDs 14, 18, 22 and 60 and for auditory startle on PNDs 23 and 61. One male or one female from each litter was tested for learning and memory in a water maze either on day 21 or 59 and then again on days 24 or 62. On PND 12, one male or one female from each litter was sacrificed; the brains were weighed 24 hours after fixation and neuropathology assessments conducted. On PND 63, one male or one female from each litter was killed; the brains were weighed, fixed and stored. Another 10 rats/sex/group (at least) were anaesthetized by a intraperitoneal injection of sodium pentobarbital and killed by perfusion fixation with formal saline for neuropathology.

TABLE 1. Study design

Experimental parameter	Dose (ppm in diet)			
	0	50	400	4000
Maternal animals				
	No. of maternal animals assigned			
FOB (GDs 10 and 17, LDs 2 and 9)	30	30	30	30
Offspring				
	No. of offspring assigned[a]			
Detailed clinical/FOB (PND 5, 12, 22, 36, 46, 61)	≥ 10/sex	≥ 10/sex	≥ 10/sex	≥ 10/sex
Motor activity (PND 14, 18, 22, 60)	≥ 10/sex	≥ 10/sex	≥ 10/sex	≥ 10/sex
Auditory startle habituation (PND 23, 61)	≥ 10/sex	≥ 10/sex	≥ 10/sex	≥ 10/sex
Learning and memory (PND 21, 24, 59, 62)	≥ 10/sex	≥ 10/sex	≥ 10/sex	≥ 10/sex
Brain weight PND 12 PND 63 PND 63 (post-perfusion)	 ≥ 10/sex ≥ 10/sex ≥ 10/sex	 ≥ 10/sex ≥ 10/sex ≥ 10/sex	 ≥ 10/sex ≥ 10/sex ≥ 10/sex	 ≥ 10/sex ≥ 10/sex ≥ 10/sex
Neuropathology PND 12 PND 63[b]	 ≥ 10/sex ≥ 10/sex			 ≥ 10/sex ≥ 10/sex

[a] At least 10 male and 10 female animals per group (one male and one female from each litter).
[b] Selected areas of the dorsal cortex, thalamus and hippocampus were processed from animals in the intermediate groups.

5. **Dose selection rationale:** Dose levels were chosen based on the results from a preliminary developmental neurotoxicity study in the rat (MRID 46028201). The study results are discussed in detail in the appendix of this DER.

6. **Dosage administration:** Thiamethoxam was administered to parent female Wistar-derived rats in the diet at levels of 0, 50, 400 or 4000 ppm from GD 7 through to PND 22; the dose rates during gestation were 0, 4.3, 34.5 and 298.7 mg/kg/day, respectively. The test substance intake was 0, 8.0, 64.0 and 593.5 mg/kg/day, respectively, during lactation.

7. **Dosage preparation and analysis:** The experimental diets were made in 20 kg batches at monthly intervals from premixes which were prepared by mixing the appropriate amount of the test material with milled CT1 diet. The premixes (one per group) were added to the appropriate quantity of the diet and mixed in a blender. The prepared diets were stored in glass jars at room temperature.

   Samples from all dietary levels were analyzed twice during the study for the quantity of thiamethoxam. The homogeneity of the test material in the diet was determined by analyzing samples from the low and high dose levels. The stability of thiamethoxam in the diet was determined for a period of 28 or 57 days for the 50 ppm and 4000 ppm levels, respectively.

Results:

Homogeneity analysis: Homogeneity was determined for six samples from the top, middle and bottom of the 50 and 4000 ppm diets. The mean concentrations were 48.1 ppm for the 50 ppm diet and 3683 ppm for the 4000 ppm test diet; the percentage deviations from the overall mean were within 5%. The analyses suggest that the thiamethoxam was adequately distributed.

Stability analysis: At nominal concentrations of 50 and 4000 ppm, thiamethoxam was stable in the diet at room temperature for 28 days and 57 days, respectively (50 ppm diet: 94.9-100% of initial; 4000 ppm diet: 93.4-104.9% of initial).

Concentration analysis: The 50, 400 and 4000 ppm dietary levels averaged 91.6%, 99.6% and 97.6% of the nominal concentration, respectively.

The analytical data indicated that the concentration, stability, and homogeneity of thiamethoxam in the diets were adequate.

C. OBSERVATIONS:

1. In-life observations:

a. **Maternal animals:** Detailed clinical observations were recorded when the rats were weighed. Cage-side observations were conducted twice daily (morning and evening).

   On GDs 10 and 17 and LDs 2 and 9, all the females were examined outside the home cage. The following functional observations were recorded.

Functional observations–Maternal animals	
X	Signs of autonomic function, including: 1) Ranking of degree of lacrimation and salivation, with range of severity scores from none to severe 2) Presence or absence of piloerection and exophthalamus. 3) Ranking or count of urination and defecation, including polyuria and diarrhea 4) Pupillary function such as constriction of the pupil in response to light, or a measure of pupil size 5) Degree of palpebral closure, e.g., ptosis.
X	Description, incidence, and severity of any convulsions, tremors, or abnormal movements.
X	Description and incidence of posture and gait abnormalities.
X	Description and incidence of any unusual or abnormal behaviors, excessive or repetitive actions (stereotypies), emaciation, dehydration, hypotonia or hypertonia, altered fur appearance, red or crusty deposits around the eyes, nose, or mouth, and any other observations that may facilitate interpretation of the data.

Individual maternal body weight was recorded immediately prior to dosing on GD 7, on GDs 15 and 22 and on LDs 1, 5, 8, 12, 15 and 22. Food consumption measurements were recorded on GDs 1, 7, 15 and 22 and on LDs 1, 5, 8, 15 and 22 and were calculated as g/rat/day.

b. **Offspring**:

1) **Litter observations:** Each litter was examined as soon as possible (always within 24 hours) after completion of parturition and was checked daily for dead or abnormal pups. Animals found dead or sacrificed for humane reasons were given a postmortem examination.

On day 5 *post partum*, litters were standardized to a maximum of 8 pups/litter (4/sex/litter, as nearly as possible); pups not selected were killed and discarded. Litters of 8 pups or less were not standardized; litters of 7 or 8 pups with at least 3 pups of each sex were used for the F_1 generation.

2) **Developmental landmarks:** Beginning on PND 29, female offspring were examined daily for vaginal patency. Beginning on PND 41, male offspring were examined daily for balanopreputial separation. The age of onset and the offspring's body weight at that time were recorded.

3) **Detailed observations:** The sex, weight and clinical condition of each offspring was recorded on days 1 and 5 *post partum*. Detailed clinical observations were recorded at the same time as body weight beginning on day 5 *post partum*. Individual offspring body weight data were recorded on PNDs 5, 12, 18, 22, 29, 36, 43, 50, 57 and prior to termination on day 63. Individual food consumption was not measured.

4) **Neurobehavioral evaluations:** Observations and the schedule for those observations are summarized as follows from the report.

I) **Functional observational battery (FOB):** On PNDs 5, 12, 22, 36, 46 and 61, at least 10 male and 10 female pups per group (one male or one female from each litter) were examined outside the home cage in an FOB assessment by observers blind to the treatment groups. The methods were similar to the procedures used for the dams.

	FUNCTIONAL OBSERVATIONS- Offspring
X	Signs of autonomic function, including: 1) Ranking of degree of lacrimation and salivation, with range of severity scores from none to severe; 2) Presence or absence of piloerection and exophthalamus; 3) Ranking or count of urination and defecation, including polyuria and diarrhea; 4) Pupillary function such as constriction of the pupil in response to light, or a measure of pupil size; 5) Degree of palpebral closure, e.g., ptosis.
X	Description, incidence, and severity of any convulsions, tremors, or abnormal movements.
X	Description and incidence of posture and gait abnormalities.
X	Description and incidence of any unusual or abnormal behaviors, excessive or repetitive actions (stereotypies), emaciation, dehydration, hypotonia or hypertonia, altered fur appearance, red or crusty deposits around the eyes, nose, or mouth, and any other observations that may facilitate interpretation of the data.

ii) **Motor activity testing:** Motor activity was evaluated in one male or one female from each litter on PNDs 14, 18, 22 and 60. Only limited information on the methodology used for assessing motor activity was described in the study report. "The test was conducted in a separate room to minimize disturbance and used automated activity recording apparatus which recorded small and large movements as an activity count. Each assessment was divided into 10 scans of five minute duration during which food, water and items of environmental enrichment were not provided. Treatment groups were counter balanced across cage/device numbers (up to 32 animals per trial/run), and when the trials were repeated, each animal was returned to the same activity monitor."

iii) **Auditory startle habituation:** Auditory startle reflex habituation testing was performed on one male or one female from each litter on PNDs 23 and 61, using an automated system.

Only limited information is provided in the study report on methodology and equipment used for the measurements. "The mean response amplitude and time to maximum amplitude on each block of 10 trials (5 blocks of 10 trials per session on each day of testing) was calculated."

iv) **Learning and memory testing:** Learning and memory testing was performed on one male and one female from each litter. A "Y" shaped water maze with one escape ladder was used for the test. The time required for a pup to find the escape ladder was recorded for each trial. The pups were given 6 trials on either PND 21 or 59. A straight "maze"

(channel) was used to evaluate swimming speed. Each animal completed one trial in this channel immediately after the six trials in the "Y" shaped water maze. Three days later (days 24 or 62) the animals were re-tested using the same procedures. The criterion for a successful trial was a time less than the cut-off values of 3, 4, 5, 6, 7, 8, 9, and 10 seconds and 1, 1.5, and 2 times the straight channel time. For each individual animal, the percentage of trials meeting a specific criterion was used to calculate the group mean for that criterion.

2. **Postmortem observations**:

a. **Maternal animals:** Maternal animals were sacrificed by carbon dioxide inhalation on PND 29. Adult females were not routinely subjected to a gross necropsy. Females with litters not selected on day 5 *post partum* and females with total litter loss were sacrificed and discarded without examination. One female which failed to litter was sacrificed. A gross necropsy was performed, including an examination of the uterus to confirm pregnancy status, but no tissues were processed for histopathology.

b. **Offspring:** Selected F_1 animals which were found dead or killed were examined macroscopically and abnormalities stored in fixatives. Pups not selected on day 5 *post partum* were killed and discarded without examination. Pups which died or were killed prior to day 5 *post partum* were given a macroscopic visceral examination and discarded.

The offspring selected for brain weight or neuropathological evaluation were sacrificed on PND 12 or 63. These animals were subjected to postmortem examinations as described below.

At PND 12, one male or one female from each litter (at least 10 of each sex per group) was sacrificed by exposure to carbon dioxide. The brains were immediately exposed, immersed in 10% neutral buffered formol saline and were weighed after approximately 24 hours of fixation. The brains from the control and 4000 ppm group were embedded in paraffin wax, sectioned into 7 levels and stained with hematoxylin and eosin. Detailed diagrams of the morphometric measurement are included in Appendix F (pages 155-161) of the study report but no text is provided to explain the procedures used.

On PND 63, one male or one female from each litter (at least 10 of each sex per group) was sacrificed by exposure to carbon dioxide. After weighing, the brain was fixed and stored. Another 10 rats/sex/dose were anaesthetized by intraperitoneal injection of pentobarbital and perfused with formol saline. The volume of fixative was approximately equivalent to estimated body weight of the pup. The brain was removed and weighed. The following tissues were preserved in an appropriate fixative:

brain
eye with optic nerve and retina*
spinal cord (including cervical and lumbar swellings)

spinal nerve roots (dorsal and ventral root fibers) at cervical swellings
spinal nerve roots (dorsal and ventral root fibers) at lumbar swellings
dorsal root ganglia at cervical swelling
dorsal root ganglia at lumbar swelling
proximal sciatic nerve*
proximal tibial nerve*
distal tibial nerve (calf muscle branches)*
gastrocnemius muscle*
*(right and left preserved; left processed for examination)

The brain was sectioned into 7 levels. Transverse sections of the following tissues were embedded in paraffin wax: gastrocnemius muscle, eye (with retina and optic nerve), spinal cord (at cervical and lumbar swellings) to include dorsal root ganglia and spinal nerve roots (dorsal and ventral root fibers). Longitudinal sections of the spinal cord at cervical and lumbar swellings were embedded in paraffin wax. Initially, tissues from control and high dose animals were sectioned and stained with hematoxylin and eosin. Subsequently, areas of the dorsal cortex, thalamus and hippocampus were processed from animals in the low and intermediate dose groups.

For all groups, transverse and longitudinal sections of the following tissues were embedded in resin: proximal sciatic nerve, proximal tibial nerve and distal tibial nerve (calf muscle branches). Tissue sections from the control and high dose animals were cut and stained with toluidine blue.

Each of the seven brain levels was embedded rostral side down in paraffin wax. The cerebellum was cut sagitally in the mid-line and the two pieces embedded, medial side down, in paraffin wax. Morphometric measurements on levels 2-5 of the brain and the section of the cerebellum were done using a KS400 image analyzer. Detailed diagrams of the brain measurements are included in Appendix F (pages 155-161) of the study report but no text is provided to explain the procedures used.

D. DATA ANALYSIS:

1. **Statistical analyses:** The following data were analyzed using an analysis of variance (ANOVA): maternal day 1 *post partum* body weight, maternal food consumption during gestation (from day 7) and *post partum*, litter size initial (day 1), mean pup body weight and total litter weight, day 5 litter-based mean body weight, motor activity measurements, max amplitude and time to maximum amplitude in startle response tests, time to preputial separation or vaginal opening, swimming times in the straight channel and brain weight. The mean percentage of successful trials at each cut-off value in the Y-maze was considered by an ANOVA following the double arcsine transformation of Freeman and Tukey, separately for males and females.

The following data were analyzed using an analysis of covariance (ANCOVA): maternal gestation and lactation body weights using GD 7 body weight and LD 1 body weight, respectively, as covariants; pup body weight after day 1 using initial body weight as covariant, and offspring brain weight with final body weight as covariant.

The proportion of whole litter losses and the proportion of males and females with developmental landmarks (preputial separation and vaginal opening) on each day were analyzed using the Fisher's exact test.

For live born pups, pre- and post-cull pup survival and pup sex, the following analyses were used: 1) percentages were considered by ANOVA following the double arcsine transformation of Freeman and Tukey; 2) the proportion of pups born alive, the proportion of pups surviving, the proportion of litters with all pups born alive, the proportion of litters with all pups surviving and the proportion of male pups were considered by Fisher's Exact Test.

Brain morphology data were analyzed by ANOVA and ANCOVA using final body weight as a covariate.

2. **Indices:**

   a. **Reproductive indices:** Standard reproductive indices were not calculated since the females were mated prior to delivery to the testing laboratory.

   b. **Offspring viability indices:** The following viability (survival) indices were reported: proportion (%) of pups born alive; proportion of litters with all pups born alive; litter size day 1; litter size day 5; proportion of whole litter losses; proportion (%) of pups surviving to day 5; proportion of litters with all pups surviving to day 5; sex ratio on days 1 and 5.

3. **Positive and historical control data:** Historical control data were provided for brain morphometric data on day 63 for the following:

Level 3 - Dorsal Cortex 1 - Thickness
Level 4 - Thalamus - Width
Level 4 - Thalamus/Cortex - Overall Width
Level 5 - Hippocampus - Width Overall
Level 5 - Thalamus Width
No positive control data were provided.

II. RESULTS:

A. PARENTAL ANIMALS:

1. **Mortality and clinical and functional observations**: One female at 4000 ppm failed to litter and was sacrificed; she was found to be non-pregnant. One female each at 50 ppm, 400 ppm and 4000 ppm had a total litter loss and was sacrificed. The following number of females were sacrificed due to insufficient pups (less than 3/sex in litter of at least 7 pups) in the control, 50 ppm, 400 ppm and 4000 ppm groups: 8, 6, 2 and 2, respectively.

   No treatment-related clinical or functional findings in females were observed during the general observations and the functional observation battery on GDs 10 and 17 and LDs 2 and 9.

2. **Body weight and food consumption**: Selected group mean body weight and food consumption values for pregnant or nursing dams are summarized in Table 2. The following were observed in females at 4000 ppm: significantly decreased (95-96% of control value) body weight during gestation; decreased (88% of control value) body weight gain during gestation; significantly decreased (80-83% of control value) food consumption during the latter part of gestation (days 7-15 and 15-22); significantly decreased (93-97% of control value) body weight beginning on LD 1 and continuing to LD 22; significantly decreased (80-91% of control value) food consumption throughout lactation. No treatment-related findings were observed in females at 50 or 400 ppm.

TABLE 2. Selected mean (±SD) maternal body weight and food consumption[a]

Observations/study interval	Dose (ppm)			
	0	50	400	4000
Gestation (n= 29-30)				
Body wt. Gestation day 1 (g)	262.0±17.6	259.1±19.4	261.9±17.4	257.9±20.8
Body wt. Gestation day 7 (g)	294.5±18.0	292.6±17.0	296.4±18.1	290.6±22.1
Body wt. Gestation day 15 (g)[b]	335.3±20.1	335.1±19.3	334.4±19.0	320.7**±21.9 (96)
Body wt. Gestation day 22 (g)[b]	417.0±25.0	415.6±27.6	417.8±23.5	394.9**±27.0 (95)
Wt. gain gestation days 1-22 (g)c	155.0	156.5	155.9	137.0 (88)
Food consumption gestation days 1-7 (g/day)	23.1±3.2	22.5±2.7	22.8±2.3	22.1±3.2
Food consumption gestation days 7-15 (g/day)	28.3±3.4	26.6*±2.4	27.3±2.2	22.7**±2.8 (80)
Food consumption gestation days 15-22 (g/day)	32.0±3.8	31.9±3.6	32.6±4.1	26.6**±3.6 (83)
Lactation (n=22-30)[d]				
Body wt. lactation day 1(g)	314.6±27.8	313.1±25.0	313.2±25.1	291.7**±24.3 (93)
Body wt. lactation day 5 (g)[b]	333.9±25.5	328.5±22.7	332.2±21.9	323.7**±22.7 (97)
Body wt. lactation day 8 (g)[b]	345.9±25.7	337.6±21.5	339.3±22.9	331.3**±25.4 (96)
Body wt. lactation day 15 (g)[b]	369.1±25.3	362.5±22.8	362.0±22.9	344.9**±24.2 (93)
Body wt. lactation day 22 (g)[b]	373.7±27.6	368.0±19.5	368.3±18.3	360.7**±21.3 (97)
Body wt. lactation day 29 (g)[b]	353.1±28.3	350.2±21.2	353.2±21.8	352.4±25.7
Food consumption lactation days 1-5 (g/day)	37.3±6.9	35.2±6.4	37.1±3.9	30.5**±5.5 (82)

TABLE 2. Selected mean (±SD) maternal body weight and food consumption[a]				
Observations/study interval	Dose (ppm)			
	0	50	400	4000
Food consumption lactation days 5-8 (g/day)	49.8±8.7	46.7±7.7	45.7±7.2	39.9**±7.5 (80)
Food consumption lactation days 8-15 (g/day)	61.7±6.3	62.5±7.8	64.1±6.1	52.5**±7.0 (85)
Food consumption lactation days 15-22 (g/day)	77.4±5.3	78.2±6.6	78.1±6.3	70.5**±6.2 (91)

[a] Data obtained from Tables 5-8, pages 51-54, MRID 46028202
[b] Mean adjusted for initial value/covariate.
[c] Calculated by the reviewer; no standard deviation calculated.
[d] Excludes whole litter losses
** Statistically significantly different from control, p≤ 0.01.
Number in parentheses is % of control value, calculated by reviewer.

3. **Test substance intake:** Based on maternal food consumption and body weight and nominal dietary concentrations, the doses expressed as mean daily mg test substance/kg body weight during the gestation and lactation periods are presented in Table 3.

TABLE 3. Mean maternal test substance intake (mg/kg body weight/day)			
Period	50 ppm	400 ppm	4000 ppm
Gestation	4.3	34.5	298.7
Lactation	8.0	64.0	593.5

Data obtained from text p. 25, MRID 46028202.

4. **Reproductive performance:** The study report states that all females littered on day 22 of gestation; therefore, there were no treatment-related effects on gestation length. Litter size and offspring survival are given below. No other data were provided.

B. **OFFSPRING:**

1. **Viability and clinical signs:** Litter size and viability (survival) results from pups during lactation are summarized in Table 4. There were no treatment-related effects on pup survival or litter size on days 1 and 5 (pre-cull). One female each in the treated groups had a whole litter loss on day 4 or 5 *post partum*. Three animals were sacrificed due to clinical signs but none were considered treatment-related. A female in the 4000 ppm group was killed on day 18 due to dry eye; a female in the 400 ppm group had a broken leg on day 36; and a male in the 4000 ppm group had a hole in the palate on day 61.

TABLE 4. Litter size and viability [a]				
Observation	Dose (ppm)			
	0	50	400	4000
Number of Litters	30	30	30	29
Total number born	361	371	390	382
Number born live	358	363	386	380
Number born dead	3	8	4	2
Whole litter losses	0/30 (0%)	1/30 (3.3%)	1/30 (3.3%)	1/29 (3.4%)
Pup Survival (days 1-5)[b,c]	344/358 (95.8%)	336/347 (96.8%)	362/372 (97.5%)	354/367 (96.8%)
Sex Ratio Day 1 (%♂)	44.8±15.8	50.4±16.7	56.6*±13.1	50.0±16.1
Mean litter size:				
Day 1	11.9±3.2	12.0±3.4	12.8±2.5	13.1±2.0
Day 5 [c]	11.5±3.4	11.6±3.4	12.5±2.4	12.6±1.7

[a]Data obtained from Table 9, pages 55-56, MRID 46028202.
[b]Before standardization (culling).
c Excludes whole litter losses

2. **Body weight**: The following were observed in male and female pups born to dams at 4000 ppm: significantly decreased body weight on days 1 (91-93% of control value) and 5 (pre-cull) (95-96% of control value) which remained lower (88-96% of control value) throughout lactation, decreased (80-87% of control value) body weight gain during lactation; and significantly decreased (92-96% of control value) body weight post-weaning. Selected mean pre-weaning pup body weight data are presented in Table 5, and selected mean post-weaning offspring body weight data are presented in Table 6.

TABLE 5. Mean (±SD) pre-weaning pup body weights and body weight gain (g) [a]

Postnatal Day	Dose (ppm)							
	0	50	400	4000	0	50	400	4000
	Males				Females			
1	6.1±0.7	5.9±0.6	6.0±0.5	5.7**±0.5 (93)	5.8±0.7	5.6±0.6	5.6±0.6	5.3**±0.4 (91)
5[b,c]	9.8±1.4	9.8±1.2	9.7±0.9	9.3**±1.0 (95)	9.3±1.4	9.3±1.2	9.2±1.0	8.9*±0.9 (96)
5[d]	10.0±1.3	9.8±1.1	9.9±0.9	8.8**±1.0 (88)	9.6±1.2	9.3±1.1	9.3±0.8	8.4**±0.9 (88)
12[e]	24.[illegible]±1.9	24.1±1.6	24.0±1.4	22.0**±1.5 (91)	23.5±1.9	23.2±1.7	23.1±1.5	21.3**±1.4 (91)
22[e]	54.3±3.3	54.2±3.7	54.1±2.8	48.5**±2.8 (89)	52.6±3.0	52.3±4.2	51.3±2.9	47.4**±2.6 (90)
Weight gain Days 1-5[e]	3.8	4.0	3.8	3.2 (80)	3.7	3.7	3.7	3.1 (84)
Weight gain Days 5-12[e]	14.5	14.5	14.4	12.5 (86)	14.4	14.1	14.0	12.2 (85)
Weight gain Days 12-22[e]	30.8	30.1	30.2	26.2 (85)	29.4	29.2	28.2	25.7 (87)

[a] Data obtained from Tables 10, 14 and unnumbered table, pages 28, 57 and 64-67 MRID 46028202.
[b] Before standardization (culling).
[c] Mean adjusted for initial value/covariate.
[d] After standardization (culling).
[e] Calculated by the reviewer.
* Statistically significantly different from control, p≤ 0.05
** Statistically significantly different from control, p≤ 0.01.
Number in parentheses is % of control value, calculated by reviewer.

TABLE 6. Mean (±SD) post-weaning pup body weights (g) [a]

Postnatal Day	Dose (ppm)							
	0	50	400	4000	0	50	400	4000
	Males				Females			
29[b]	95.4±5.4	94.0±5.6	94.3±5.0	87.5**±4.5 (92)	88.4±4.8	87.6±5.2	86.5±4.9	82.0**±3.6 (93)
36[b]	152.6±7.6	152.1±8.7	152.4±6.9	141.0**±7.1 (92)	132.7±6.3	132.7±7.9	131.2±6.8	125.4**±6.1 (94)
50[b]	272.2± 13.2	268.3± 15.3	270.8± 12.1	254.1**± 12.0 (93)	195.0± 9.8	191.8± 9.7	190.5± 9.3	184.7**± 11.0 (95)
57[b]	327.2± 17.7	323.8± 16.0	326.7± 13.8	308.3**± 14.4 (94)	217.0± 13.5	211.9± 11.5	210.8± 12.3	204.7**± 13.8 (94)
63[b]	365.0± 19.7	360.2± 19.5	363.5± 16.3	343.2**± 16.3 (94)	225.9± 15.6	220.1± 12.8	219.2± 12.8	216.1*± 15.9 (96)

[a] Data obtained from Table 14, pages 64-67, MRID 46028202.
[b] Mean adjusted for initial value/covariate.
* Statistically significantly different from control, p≤ 0.05
** Statistically significantly different from control, p≤ 0.01.
Number in parentheses is % of control value, calculated by reviewer.

3. **Developmental landmarks:**

a. **Sexual maturation:** Preputial separation in males at 4000 ppm was delayed by an average of 1.5 days in comparison to control males. Body weight was also decreased in the 4000 ppm males on the day of preputial separation. There was no treatment-related effect on the day of vaginal opening in females. The data are presented in Table 7.

Table 7. Mean (±SD) Age of Sexual Maturation (days)[a]

Parameter	Dose (ppm)			
	0	50	400	4000
N (M/F)	22/22	23/23	27/27	26/26
Preputial separation (males)	44.9±0.9	45.6*±0.8	45.1±0.9	46.4**±1.3
Body weight at landmark (g) in males	230.2±10.1	233.0±12.2	230.5±12.7	220.7**±9.7 (96)
Vaginal opening (females)	36.6±2.4	37.7±2.6	37.0±2.1	37.4±1.6

[a] Data obtained from Table 15, pages 68-69, MRID 46028202.
** Statistically significant from control value, p<0.01.
Number in parentheses is % of control value, calculated by reviewer.

b. **Developmental landmarks:** Other developmental endpoints, such as tooth eruption, pinna unfolding, etc., were not monitored.

4. **Behavioral assessments:**

a. **Functional observational battery:** There were no treatment-related effects on offspring on any test day (PNDs 5, 12, 22, 36, 46 and 61).

b. **Motor/locomotor activity:** No treatment-related overall or interval motor activity effects were noted. Habituation was apparent in all groups by PND 22. Total activity data are presented in Table 8.

TABLE 8. Mean (±S.D.) motor activity data (total activity counts for session) [a]				
Test Day	Dose (ppm)			
	0	50	400	4000
Males (n=11-13)				
PND 14	182.7±110.7	69.0*±60.3	108.6±126.7	107.1±113.7
PND 18	189.4±148.3	122.6±120.1	224.0±174.2	186.3±159.3
PND 22	415.2±126.3	345.0±156.8	467.6±167.6	452.0±154.5
PND 60	490.6±127.8	512.4±154.0	498.7±138.6	601.3±166.4
Females (n=11-14)				
PND 14	80.9±92.2	154.6±124.0	113.7±89.6	183.4±175.8
PND 18	219.4±157.3	201.3±125.6	167.5±146.3	261.6±200.2
PND 22	423.4±124.6	433.9±136.9	376.6±144.8	435.6±171.5
PND 60	546.7±146.3	572.9±142.9	606.7±81.5	611.9±94.0

[a] Data obtained from Table 16, pages 70-77, MRID 46028202.
* Statistically significantly different from control, $p \leq 0.05$

c. **Auditory startle reflex** : There were no treatment-related effects in males or females on startle amplitude or time to maximum amplitude on any test day. Habituation was apparent on both testing days in both sexes with successive repetitions. Peak amplitude data are summarized in Table 9 and time to maximum amplitude data are summarized in Table 10

TABLE 9. Auditory startle amplitude (v) (mean ±SD) [a]

	Trial Block	Dose (ppm)			
		0	50	400	4000
Males (n=11-13)					
PND 23	1	604.0±354.7	382.9*±198.2	405.3*±148.9	429.6±144.4
	2	362.9±195.4	274.2±106.9	290.5±74.6	338.0±152.5
	3	291.4±78.7	265.6±114.5	285.9±80.7	268.2±75.8
	4	239.5±41.7	268.7±82.3	269.1±107.3	241.6±87.0
	5	230.7±48.5	236.0±79.6	253.1±72.2	234.9±59.5
PND 61	1	1246.8±531.9	1165.7±331.1	1302.4±517.0	1295.9±350.2
	2	715.8±278.7	846.2±250.6	1048.1*±449.0	930.9±218.6
	3	664.6±287.4	746.2±190.1	970.5*±446.8	858.5±241.8
	4	596.5±181.3	731.5±200.4	931.8**±348.2	761.5±196.0
	5	709.8±211.6	700.3±180.1	884.7±383.2	792.9±257.4
Females (n=11-14)					
PND 23	1	600.8±387.1	450.2±178.3	533.9±220.3	377.1*±144.9
	2	345.2±130.8	344.3±137.6	352.1±82.5	272.4±67.7
	3	263.9±103.3	364.1±197.2	335.4±114.3	251.6±56.9
	4	276.6±78.6	293.1±126.7	316.6±149.9	220.1±67.5
	5	271.6±98.2	267.7±92.7	291.9±128.0	214.1±56.9
PND 61	1	1021.6±251.1	1104.4±361.2	1148.5±608.1	862.5±246.2
	2	896.8±282.5	932.7±262.7	844.8±210.7	810.0±293.4
	3	803.2±294.7	700.5±288.5	778.3±237.5	668.4±220.0
	4	685.0±230.3	748.3±297.9	766.5±230.6	626.8±203.7
	5	789.0±228.5	717.0±252.2	802.5±263.7	627.3±188.4

[a]Data obtained from Table 17, pages 78-81. MRID 46028202
* Statistically significantly different from control value, $p<0.05$
** Statistically significantly different from control value, $p<0.01$

TABLE 10. Auditory startle: time to peak amplitude (mean msec ±S.D.) [a]

	Trial Block	Dose (ppm)			
		0	50	400	4000
Males (n=11-13)					
PND 23	1	29.2±7.9	27.5±4.1	26.0±4.3	27.9±7.2
	2	22.2±4.7	22.9±3.3	20.6±2.0	22.9±8.1
	3	21.2±3.9	23.4±6.3	20.4±3.2	19.6±1.6
	4	20.3±1.9	21.7±2.3	20.8±2.3	19.9±1.6
	5	19.5±1.3	20.4±2.3	19.4±1.3	19.3±1.7
PND 61	1	29.0±4.2	25.0±2.5	25.5±7.9	26.4±4.1
	2	25.2±4.5	22.7±2.5	24.9±7.7	22.5±3.5
	3	25.0±4.0	22.2*±2.6	22.6±3.4	21.8*±2.1
	4	25.0±2.4	23.0±2.4	23.2±3.3	23.7±3.9
	5	23.5±2.2	23.3±2.9	24.2±3.0	23.6±3.5
Females (n=11-14)					
PND 23	1	30.1±9.6	27.8±7.5	28.1±6.3	28.8±6.2
	2	23.4±9.8	25.2±8.2	22.2±4.0	20.4±2.3
	3	21.2±2.4	25.0±12.9	21.1±4.4	19.7±1.6
	4	20.8±2.5	22.4±6.8	21.2±4.0	21.1±3.2
	5	20.6±2.7	22.5±9.9	22.2±4.2	19.8±1.4
PND 61	1	24.0±5.5	23.2±3.2	25.2±5.2	26.4±4.1
	2	23.7±4.2	24.1±3.5	23.1±4.5	22.9±3.2
	3	22.5±2.7	22.7±2.6	23.4±4.4	23.7±5.7
	4	23.7±2.7	23.6±2.9	22.9±3.7	23.1±3.3
	5	21.7±2.9	22.3±2.8	22.5±1.9	24.2*±3.3

[a]Data obtained from Table 18, pages 82-85. MRID 46028202.
* Statistically significantly different from control value, $p<0.05$

d. **Learning and memory testing:**

Water Maze: There were no treatment-related differences for males or females at any dose level compared to controls with regard to learning and memory. Data are summarized in Table 11.

TABLE 11. Selected water maze performance parameters[a]

Test Day/Parameter		Dose (ppm)			
		0	50	400	4000
Males (n=19-27)					
Day 21 (Learning)	Straight channel (sec) (mean ± SD)	5.8±2.5	5.7±3.0	5.0±2.7	4.6±3.5
	Straight channel -≤2.0x (%)	54.4±28.8	47.7±33.5	51.9±30.1	48.0±25.1
	Trial 1 duration (sec) (mean ± SD)	18.2±8.8	16.4±6.5	15.6±8.6	15.0±6.3
	Trial 6 duration (sec) (mean ± SD)	7.0±4.6	9.5±4.9	7.7±5.6	7.5±3.4
	Successful trials - ≤ 10 secs (%)	48.2±20.7	47.0±27.5	61.7±23.5	62.0±18.3
Day 24 (memory)	Straight channel (sec) (mean ± SD)	4.7±2.2	3.8±1.6	3.7±1.1	3.7±1.8
	Straight channel -≤2.0x (%)	82.5±21.1	75.0±22.9	77.2±22.7	64.7**±25.6
	Trial 1 duration (sec) (mean ± SD)	10.2±6.0	9.5±4.2	9.9±5.7	9.0±4.5
	Trial 6 duration (sec) (mean ± SD)	7.0±4.6	4.3*±2.0	5.8±3.6	7.1±5.0
	Successful trials - ≤ 10 secs (%)	87.7±10.9	87.9±13.8	85.8±13.6	88.0±14.8
Day 59 (learning)	Straight channel (sec) (mean ± SD)	4.6±1.6	4.6±1.4	4.5±1.9	4.1±1.2
	Straight channel -≤2.0x (%)	77.0±17.1	81.8±11.4	71.8±17.5	72.7±20.9
	Trial 1 duration (sec) (mean ± SD)	13.6±4.1	16.2±7.4	14.2±5.6	13.4±5.6
	Trial 6 duration (sec) (mean ± SD)	5.4±2.8	4.8±1.9	5.3±3.1	4.5±1.9
	Successful trials - ≤ 10 secs (%)	81.7±9.0	83.3±11.5	80.1±11.6	80.0±17.3
Day 62 (memory)	Straight channel (sec) (mean ± SD)	4.4±2.4	4.2±2.0	4.1±2.4	4.2±1.7
	Straight channel -≤2.0x (%)	67.5±27.1	75.0±27.6	73.3±24.1	72.2±23.4
	Trial 1 duration (sec) (mean ± SD)	5.2±2.4	6.1±5.2	5.7±3.0	5.0±2.3
	Trial 6 duration (sec) (mean ± SD)	10.3±9.2	8.2±8.3	6.7±4.9	7.2±6.0
	Successful trials - ≤ 10 secs (%)	75.4±22.7	86.4±22.2	90.7*±18.1	80.6±18.8
Females (n=21-27)					
Day 21 (learning)	Straight channel (sec) (mean ± SD)	4.7±1.9	5.0±.2.2	4.0±0.9	4.8±2.0
	Straight channel -≤2.0x (%)	50.8±21.5	58.7±26.2	48.1±23.3	44.0±28.8
	Trial 1 duration (sec) (mean ± SD)	14.3±4.8	17.0±8.3	14.5±5.5	17.9±7.4
	Trial 6 duration (sec) (mean ± SD)	8.5±4.7	9.7±7.8	6.2±3.4	9.0±6.1
	Successful trials - ≤ 10 secs (%)	56.8±19.7	60.3±23.3	64.2±19.4	50.0±22.6
Day 24 (memory)	Straight channel (sec) (mean ± SD)	3.8±1.1	3.9±1.8	4.3±2.9	3.3±0.9
	Straight channel -≤2.0x (%)	65.9±26.5	74.6±18.0	74.7±18.1	68.7±20.6
	Trial 1 duration (sec) (mean ± SD)	9.0±5.0	9.3±4.1	9.1±5.2	9.0±3.4
	Trial 6 duration (sec) (mean ± SD)	7.6±4.9	6.9±5.7	5.1±3.1	7.3±7.4
	Successful trials - ≤ 10 secs (%)	75.8±24.0	86.5±11.3	84.0±12.6	82.7±18.3
Day 59 (learning)	Straight channel (sec) (mean ± SD)	5.2±3.2	4.2±1.2	5.0±3.9	4.5±2.3
	Straight channel -≤2.0x (%)	80.3±12.2	78.6±12.0	74.7±16.7	72.9±17.6
	Trial 1 duration (sec) (mean ± SD)	13.4±3.9	16.1±7.3	13.3±3.5	15.7±5.0
	Trial 6 duration (sec) (mean ± SD)	4.9±3.5	4.5±2.5	5.4±3.2	4.8±2.6
	Successful trials - ≤ 10 secs (%)	80.3±8.4	82.5±12.3	79.3±16.9	77.1±16.2
Day 62 (memory)	Straight channel (sec) (mean ± SD)	3.6±0.9	4.0±2.3	3.5±1.0	3.8±1.6
	Straight channel -≤2.0x (%)	64.4±22.0	72.2±23.8	63.3±26.8	61.1±27.7
	Trial 1 duration (sec) (mean ± SD)	4.0±1.6	4.4±2.1	4.5±1.8	5.1±2.7
	Trial 6 duration (sec) (mean ± SD)	9.5±6.1	7.5±6.1	9.3±8.0	9.9±9.1
	Successful trials - ≤ 10 secs (%)	78.0±21.4	82.5±20.1	75.3±26.8	79.9±23.6

[a]*Data obtained from* Tables 19-20, pages 86-109, MRID 46028202

* Statistically significantly different from control value, $p<0.05$

** Statistically significantly different from control value $p<0.01$

5. **Postmortem results:**

a. **Brain weights:** Mean brain weight data are presented in Table 12. Absolute brain weight was statistically significantly lower (95-98% of control value) in males and females at 4000 ppm on days 12 and 63. After perfusion on day 63, brain weight was significantly lower in males and females at 4000 ppm (95% of control value) and females at 400 ppm (96% of control value). These deceases were determined to be treatment related.

TABLE 12. Mean (±SD) Brain Weight Data in Offspring [a]

Parameter	Dose (ppm)			
	0	50	400	4000
Males				
Day 12 (n=11-14)				
Terminal body weight (g)	23.5±1.3	24.8±1.5	24.8±1.7	20.7±1.6 (88)
Brain weight (g)	1.15±0.04	1.16±0.04	1.13±0.04	1.10*±0.05 (96)
Day 63 (n=11-13)				
Terminal body weight (g)	372.3±18.8	361.1±28.2	370.8±20.8	346.8±22.8 (93)
Brain weight (g)	2.07±0.09	2.02±0.06	2.04±0.07	1.99*±0.09 (96)
Day 63 (Post-perfusion) (n=11-12)				
Terminal body weight (g)	369.4±19.5	363.5±32.2	355.4±26.6	341.6±11.2 (92)
Brain weight (g)	2.03±0.12	2.01±0.15	2.00±0.08	1.93*±0.07 (95)
Brain-to-body weight ratio (%)	0.55±0.02	0.55±0.03	0.57±0.05	0.56±0.03
Females				
Day 12 (n=10-13)				
Terminal body weight (g)	24.0±2.7	22.9±2.4	23.0±2.5	20.5±1.9 (85)
Brain weight (g)	1.11±0.05	1.09±0.05	1.10±0.04	1.06*±0.04 (95)
Day 63 (n=11-12)				
Terminal body weight (g)	233.4±19.8	221.8±21.2	222.5±16.3	218.7±14.1 (94)
Brain weight (g)	1.90±0.09	1.89±0.07	1.86±0.07	1.86±0.08 (98)
Day 63 (Post-perfusion) (n10-12)				
Terminal body weight (g)	227.6±17.0	220.3±17.9	217.6±13.5	206.6±19.9 (91)
Brain weight (g)	1.89±0.10	1.89±0.07	1.82*±0.06 (96)	1.80*±0.08 (95)

[a] Data obtained from Table 21, pages 110-112, MRID 46028202
* Statistically significantly different from control value, $p<0.05$
Number in parentheses is % of control value, calculated by reviewer

b) Neuropathology

1) Macroscopic examination: Animals examined on PND 12 did not demonstrate any treatment related macroscopic abnormalities. On PND 63, one high dose female was noted to have a dilated ventricle (not noted which one). Single males in the mid and high doses were noted to have renal pelvic dilation.

2) Microscopic examination: The qualitative histopathological findings are presented in Table 13. Animals examined on PND 12 did not demonstrate any treatment related microscopic abnormalities. At study termination several control and high dose animals were noted to have demyelination of the tibial or sciatic nerves. Given the consistency of this finding between treated and control animals, this was not considered to be a treatment related effect. Similarly, one high dose male had glial cell proliferation which was not considered to be the result of treatment.

Table 12. Histopathology Findings a

Parameter	Dose (PPM)			
	Control	50	400	4000
Males				
Termination				
glial cell proliferation	0/11	0/12	0/11	1/12
proximal sciatic nerve demyelination	6/11	-	-	8/12
proximal tibial nerve demyelination	5/11	-	-	6/12
Females				
Termination				
proximal sciatic nerve demyelination	1/11	-	-	3/10
proximal tibial nerve demyelination	3/11	-	-	5/10

a Data extracted from pages (117-119) of the study report.

3) Brain Morphometry:

Brain morphometric evaluation presented in Table 14 revealed the following: High dose males and females had a variety of brain measurement parameters that were less than the respective controls at PND 12 and at termination. Males on day 12 had 4 - 6% reductions (not statistically significant) in cortex height and width, hippocampus length, thalamus width and thickness of the outer granular layer of the cerebellum. The thickness of the inner granular layer of the cerebellum was increased 6% at PND 12. The thickness of the molecular layer of the cerebellum (-12.2%) and the length of the cerebellum (16.8%) were reduced significantly in high dose males on PND 12.

At the study termination high dose males had significant reductions in dorsal (↓11.4%) and piriform cortex thickness (↓9.2%), corpus callosum thickness (↓19.6%), thalamus height (↓11%) and width (↓7.6%), thalamus/cortex overall width (↓5%), and hippocampus width (↓6.5%). Dorsal cortex thickness was significantly reduced in low and mid dose males, however, there was no dose response relationship. The terminal body weights of high dose males were decreased 6.8% compared to controls. The study authors conducted an adjustment for morphometric measurements based on body weight, to discount a number of these occurrences. Although reduced body weights would be expected to play a minor role in the morphometric changes noted in high dose males, such body weight reductions (6.8%) could not account for the large brain morphometric changes noted in the high dose animals.

Females on day 12 had 4 - 15.5% reductions (not statistically significant) in corpus callosum thickness, hippocampus length, thalamus height, and thalamus/cortex overall width. Increases in the thickness of the outer and inner granular cell layers of the cerebellum (3.1 - 11.5%), and in the height and width of the frontal cortex (3.1 - 3.8%) were noted in high dose females at PND 12 as well. The thickness of the thalamus (↓6.1%) was reduced significantly in high dose females on PND 12.

At the study termination high dose females had significant reductions in dorsal (↓5.7%) cortex thickness, thalamus width (↓7.6%), thalamus/cortex overall width (↓6.8%) and hippocampus width (↓5.8%). Non-significant decreases in the thickness of the molecular layer of the cerebellum (↓6.0%), and hippocampus length (↓3%), along with an increase in the thickness of the inner granular layer of the cerebellum (↑8.8%) were also noted. Terminal body weights of females were depressed 6.3% compared to controls. The study authors conducted an adjustment for morphometric measurements based on body weight, to discount a number of these occurrences. However, it is not OPP policy to adjust brain morphometry by body weight changes.

Subsequent to review of this study, HED requested that additional measurements in the two lower dose groups be conducted whenever changes in the high-dose group were significant at the 0.05 level, as well as at the 0.01 level as previously conducted, or in brain regions contiguous to a section with substantial change. These measurements are shown in Table 15. The brain morphometric changes in low- and mid-dose groups were not considered treatment related because statistically significant differences between concurrent controls and low- and mid-dose groups were sporadic and did not show consistent dose-response relationship. In addition, concurrent controls for most brain regions were on the high end of the historical control values and in some cases exceeded them. (March 6, 2007 memo, TXR # 0054519)

TABLE 14. Mean (±SD) Morphometric Data [a]

Parameter	Control	50 ppm	400 ppm	4000 ppm
Males (% of control)				
Day 12				
Frontal cortex height (mm)	5.82 ± 0.49	-	-	5.58 ± 0.71(95.9%)
Frontal cortex width (mm)	4.52 ± 0.39	-	-	4.35 ± 0.44 (96.2%)
Hippocampus length (mm)	2.72 ± 0.33	-	-	2.57 ± 0.37(94.5%)
Dorsal cortex thickness (mm)	1.21 ± 0.07	-	-	1.16 ± 0.10 (95.9%)
Thalamus width (mm)	7.54 ± 0.43	-	-	7.37 ± 0.47 (97.7%)
Thalamus/cortex overall width (mm)	12.51 ± 0.50	-	-	12.04 ± 0.65 (96.2%)
Cerebellum inner granular layer thickness (µm)	130 ± 21	-	-	139 ± 16 (106.9%)
Cerebellum outer granular layer thickness (µm)	48.8 ± 6.2	-	-	47.5 ± 7.0 (97.3%)
Cerebellum molecular layer thickness (µm)	63.8 ± 9.0	-	-	56.0 ± 6.1* (87.8%)
Cerebellum length (mm)	4.41 ± 0.32	-	-	4.11 ± 0.29* (93.2%)
Termination				
Frontal cortex height (mm)	6.76 ± 0.41	-	-	6.44 ± 0.44 (95.3%)
Dorsal cortex thickness (mm)	1.58 ± 0.12	1.35 ± 0.11**	1.37 ± 0.12**	1.40 ± 0.11** (88.6%)
Piriform cortex thickness (mm)	1.52 ± 0.18	-	-	1.38 ± 0.12* (90.8%)
Corpus callosum thickness (mm)	0.46 ± 0.06	-	-	0.37 ± 0.09* (80.4%)
Thalamus height (mm)	5.64 ± 0.46	-	-	5.02 ± 0.47* (89.0%)
Thalamus width (mm)	8.11 ± 0.51	8.04 ± 0.26	7.93 ± 0.18	7.49 ± 0.39** (92.4%)
Thalamus/cortex overall width (mm)	14.82 ± 0.66	-	-	14.08 ± 0.60* (95.0%)
Hippocampus width (mm)	1.55 ± 0.12	1.54 ± 0.10	1.61 ± 0.07	1.45 ± 0.15* (93.5%)
Cerebellum molecular layer thickness (µm)	213.8 ± 23.0	-	-	202.1 ± 18.1 (94.5%)
Females (% of control)				
Day 12				
Frontal cortex height (mm)	5.76 ± 0.59	-	-	5.94 ± 0.46 (103.1%)
Frontal cortex width (mm)	4.50 ± 0.44	-	-	4.67 ± 0.35 (103.8%)
Thalamus width (mm)	7.68 ± 0.54	-	-	7.21 ± 0.31* (93.9%)
Corpus callosum thickness (mm)	0.58 ± 0.17	-	-	0.49 ± 0.11 (84.5%)
Thalamus height (mm)	5.10 ± 0.32	-	-	4.92 ± 0.37 (96.5%)
Thalamus/cortex overall width (mm)	12.50 ± 0.73	-	-	12.02 ± 0.57 (96.2%)
Hippocampus length (mm)	4.00 ± 0.38	-	-	3.81 ± 0.28 (95.3%)

TABLE 14. Mean (±SD) Morphometric Data [a]

Parameter	Control	50 ppm	400 ppm	4000 ppm
Cerebellum inner granular layer thickness (µm)	122 ± 23	-	-	136 ± 19 (111.5%)
Cerebellum outer granular layer thickness (µm)	44.7 ± 4.0	-	-	46.1 ± 5.7 (103.1%)
Termination				
Dorsal cortex thickness (mm)	1.41 ± 0.06	1.39 ± 0.07	1.35 ± 0.07	1.33 ± 0.08* (94.3%)
Thalamus width (mm)	7.88 ± 0.34	7.651 ± 0.32	7.74 ± 0.41	7.28 ± 0.31** (92.4%)
Thalamus/cortex overall width (mm)	14.49 ± 0.50	14.41 ± 0.59	14.72 ± 0.72	13.50 ± 0.53** (93.2%)
***Hippocampus* length (mm)**	4.04 ± 0.40	-	-	3.92 ± 0.54 (97.0%)
Hippocampus width (µm)	1.55 ± 0.08	-	-	1.46 ± 0.08* (94.2%)
Cerebellum molecular layer thickness (µm)	211.7 ± 21.7	-	-	199.0 ± 12.5 (94.0%)
Cerebellum inner granular layer thickness (µm)	148 ± 27	-	-	161 ± 22 (108.8%)

a Data extracted from pages (120-143) of the study report.
N = 7 - 12 animals / dose group. * Statistically different from control, $p<0.05$. ** Statistically different from control, $p<0.01$

THIAMETHOXAM – Morphometric analysis

TABLE 15. Additional Morphometric Data[a] Mean (±SD)

BRAIN REGION	DOSE (PPM)				HISTORICAL CONTROL RANGE[§]
	0	50	400	4000	
Level 4- Corpus callosum – Thickness	0.57 ± 0.11	0.53 ± 0.06 (17%)	0.58 ± 0.05	0.55 ± 0.06 (14%)	0.528 ± 0.085 to 0.687 ±0.12
Level 4 – Thalamus Width	7.54 + 0.43	7.22 ± 0.22 (14%)	7.49 + 0.23	7.37 ± 0.47	7.48 + 0.36 to 8.35 ± 0.57
Cerebellum – length	4.41 ± 0.32	4.22 + 0.15	4.22 ± 0.32	4.11 ± 0.29*(17%)	3.71 + 0.31 to 4.45 ± 0.14
Cerebellum – Prepyramidal Fissure –Thickness of molecular layer	63.8 ± 9.0	61.0 ± 6.9	63.6 ± 4.4	56.0 ± 6.1** (112%)	45.4 ± 10.8 to 76.8 ± 14.6
Level 4- Thalamus width	7.68 ± 0.54	7.24 ± 0.26* (16%)	7.34 ± 0.32* (14%)	7.21 ± 0.31** (16%)	7.48 ± 0.28 to 8.30 ± 0.2
Level 4 – Corpus callosum – thickness	0.58 ± 0.17	0.57 ± 0.05	0.56 ± 0.06	0.49 ± 0.11 (116%)	0.49 ± 0.11 to 0.65 ± 0.09
Cerebellum – Length	4.23 ± 0.39	4.19 ± 0.23	4.17 ± 0.23	4.26 ± 0.17	3.63 ± 0.58 to 4.37 ± 0.4
Cerebellum – Prepyramidal Fissure – Thickness of molecular layer	60.9 ± 15.5	62.7 ± 7.3	62.0 ± 6.4	58.9 ± 7.9	48.1 ± 14.7 to 79.9 ± 12.8
Level 3- Dorsal Cortex 1 - Thickness[¶]	***1.58 ± 0.12***	***1.35 ± 0.11** (115%)***	***1.37 ± 0.12** (113%)***	***1.40 ± 0.11** (111%)***	***1.22 ± 0.11 to 1.53 ± 0.11***
Level 3 – Dorsal Cortex 2 – Thickness	1.88 ± 0.14	1.87 ± 0.10	1.87 ± 0.10	1.74 ± 0.16** (17%)	1.48 ± 0.09 to 1.77 ± 0.11
Level 3 – Pyriform Cortex – thickness	1.52 ± 0.18	1.51 ± 0.06	1.49 ± 0.03	1.38 ± 0.12** (19%)	1.05 ± 0.11 to 1.38 ± 0.09

TABLE 15. Additional Morphometric Data[a] Mean (±SD)

BRAIN REGION	DOSE (PPM)				HISTORICAL CONTROL RANGE[b]
	0	50	400	4000	
Level 4 – Dorsal Cortex Thickness	1.53 ± 0.16	1.46 ± 0.08 (15%)	1.46 ± 0.08 (15%)	1.36 ± 0.09** (111%)	1.11 ± 0.17 to 1.53 ± 0.16
Level 4 - Corpus Callosum Thickness	0.46 ± 0.06	0.41 ± 0.04 (111%)	0.44 ± 0.04 (111%)	0.37 ± 0.09** (120%)	0.31 ± 0.08 to 0.46 ± 0.11
Level 4 – Thalamus Height	5.64 ± 0.46	5.37 ± 0.24 (15%)	5.55 ± 0.28 (12%)	5.02 ± 0.47** (111%)	5.03 ± 0.26 to 5.42 ± 0.34
Level 4- Thalamus – width	8.98 ± 0.55	8.73± 0.34	8.58 ± 0.22*(14%)	8.39 ± 0.31** (17%)	7.48 ± 0.36 to 8.37 ± 0.38
Level 4 – Thalamus Cortex	14.82 + 0.66	14.14 + 0.69* (15%)	14.18 + 0.44* (14%)	14.08 ± 0.60* (15%)	14.2 ± 0.5 to 14.7 + 0.6
Level 4 – Hippocampus Dentate Gyrus – Width	0.64 + 0.05	0.61 + 0.05 (15%)	0.61 + 0.03 (15%)	0.58 + 0.05** (19%)	0.54 ± 0.05 to 0.64 ± 0.07
Level 5 – Dorsal Cortex	1.4 ± 0.07	1.43 ± 0.10	1.42 ± 0.07	1.32 ± 0.12* (16%)	1.19 ± 0.1 to 1.39 ± 0.13
Level 5 - Thalamus -- Width	***8.11 ± 0.51***	***8.04 ± 0.26***	***7.93 ± 0.18***	***7.49 ± 0.39** (18%)***	***7.41 ± 0.39 to 7.98 ± 0.25***
Level 5 – Hippocampus – Width	***1.55 ± 0.12***	***1.54 ± 0.10***	***1.61 ± 0.07***	***1.45 ± 0.15*(16%)***	***1.31 ± 0.11 to 1.54 ± 0.08***
[redacted]					
Level 3 – Dorsal Cortex1 – Thickness	1.51 ± 0.10	1.50 ± 0.10	1.48 ± 0.09	1.46 ± 0.09	1.22 ± 0.1 to 1.46 ± 0.11
Level 3 – Dorsal Cortex2 – Thickness	1.78 ± 0.13	1.64 ± 0.12** (18%)	1.71 ± 0.13	1.71 ± 0.11	1.47 ± 0.06 to 1.73 ± 0.12
Level 3 – Piriform Cortex - Thickness	1.41 ± 0.14	1.41 ± 0.03	1.39 ± 0.05	1.40 ± 0.14	1.09 ± 0.12 to 1.37 ± 0.15

TABLE 15. Additional Morphometric Data[a] Mean (±SD)

BRAIN REGION	DOSE (PPM)				HISTORICAL CONTROL RANGE[b]
	0	50	400	4000	
Level 4 Dorsal Cortex – Thickness	1.41 ± 0.12	1.42 ± 0.04	1.41 ± 0.05	1.29 ± 0.11** (19%)	1.16 ± 0.09 to 1.43 ± 0.09
Level 4 – Corpus callosum – Thickness	0.39 ± 0.08	0.40 ± 0.04	0.39 ± 0.05	0.42 ± 0.09	0.30 ± 0.05 to 0.45 ± 0.10
Level 4- Thalamus – Height	5.27 ± 0.38	5.60 ± 0.24(16%)	5.40 ± 0.46	5.17 ± 0.53	4.88 ± 0.43 to 5.42 ± 0.22
Level 4 – Thalamus - Width	8.46 ± 0.27	8.51 ± 0.26	8.73 ± 0.20*(13%)	8.01 ± 0.35** (15%)	8.19 ± 0.48 to 8.71 ± 0.40
Level 4 – Thalamus/cortex – width	***14.49 ± 0.50***	***14.41 ± 0.59***	***14.72 ± 0.72***	***13.50 ± 0.53** (17%)***	***13.6 ± 0.8 to 14.6 ± 0.2***
Level 4 – Hippocampus Dentate Gyrus – Width	0.61 ± 0.06	0.59 ± 0.02	0.60 ± 0.02	0.58 ± 0.07	0.49 ± 0.04 to 0.62 ± 0.02
Level 5 – Dorsal cortex – Thickness	***1.41 ± 0.06***	***1.39 ± 0.07***	***1.35 ± 0.07***	***1.33 ± 0.08** (16%)***	***1.19 ± 0.09 to 1.34 ± 0.07***
Level 5 – Thalamus –width	***7.88 ± 0.34***	***7.65 ± 0.32***	***7.74 ± 0.41***	***7.28 ± 0.31** (18%)***	***7.18 ± 0.35 to 7.72 ± 0.36***
Level 5 – Hippocampus – width	1.55 ± 0.08	1.50 ± 0.03	1.55 ± 0.04	1.46 ± 0.08** (16%)	1.34 ± 0.06 to 1.58 ± 0.09

a From MRID 47034201, measurements made after the original study was conducted.
* $p < 0.05$ ** $p < 0.01$
[b] Historical control data obtained between 10/2001 and 10/2004. Concurrent control from this study not included as part of the range
[c] Italicized bolded data previously available in DER

III. DISCUSSION and CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS:** The investigators concluded that evidence of maternal toxicity at 4000 ppm included lower body weight and food consumption throughout gestation and *post partum* and lower pup body weight at birth.

Offspring born to dams at 4000 ppm remained smaller *post partum*. The slight delay in preputial separation resulting from lower body weight was considered too small to be of biological significance. Small changes in brain morphometry measurements in males and females at 4000 ppm on day 63 occurred in the absence of quantitative histopathological or behavioral findings and were considered of no toxicological significance. The NOAEL for developmental neurotoxicity was 4000 ppm.

B. **REVIEWER COMMENTS:** There were no maternal deaths or treatment-related findings during the general observations and the FOB battery tests. The following were observed in females at 4000 ppm: significantly decreased (95-96% of control value) body weight during gestation; decreased (88% of control value) body weight gain during gestation; significantly decreased (80-83% of control value) food consumption during the latter part of gestation (days 7-9 and 15-22); significantly decreased (93-97% of control value) body weight beginning on LD 1 and continuing to LD 22; and significantly decreased (80-91% of control value) food consumption throughout lactation. No treatment-related findings were observed in females at 50 or 400 ppm.

Treatment had no adverse effects on offspring survival, clinical signs, FOB, motor activity, auditory startle reflex, learning and memory, or neuropathology.

No treatment-related effects were seen on body weight, body weight gain, food consumption, brain weights or brain morphology at the low and the mid dose groups. At the high dose (4000 ppm), body weight of male and female pups were decreased on Days 1 (91-93% of control value) and Day 5 (pre-cull) (95-96% of control value) which remained lower (88-96% of control value) throughout lactation. Body weight gain were decreased (80-87% of control value) during lactation. Also at the high dose, the mean age for preputial separation was significantly delayed in male pups. Absolute brain weight was statistically significantly lower in males and females at 4000 ppm on days 12 and 63. On day 12, the length and width of the cerebellum was significantly lower in males at 4000 ppm. On day 63, significant decreases in Level 3-5 measurements were observed in males and in Level 4-5 in females at 4000 ppm.

The maternal toxicity LOAEL was 4000 ppm (298.7 mg/kg/day) based on decreases in body weight gain and food consumption. The maternal NOAEL was 400 ppm (34.5 mg/kg/day).

The offspring LOAEL was 4000 ppm (298.7 mg/kg/day) based on decreased body weight and body weight gain in males and females, delayed sexual maturation in males, and reduced brain weight and size in males and females. The offspring NOAEL was 400 ppm (34.5 mg/kg/day).

This study is classified **Acceptable/Non Guideline** and may be used for regulatory purposes. **It does not, however, satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft) pending comprehensive review of the positive control data.**

C. **STUDY DEFICIENCIES:**. Procedures and equipment used for measurements of many parameters were not described in sufficient detail.

APPENDIX: Preliminary Developmental Neurotoxicity Study

STUDY TYPE: Developmental Neurotoxicity Study - Rat; OPPTS 870.6300 (§83-6); OECD 426

PC CODE: 060109
DP BARCODE: D294153
TXR#: 0052145
SUBMISSION NO.: N/A

TEST MATERIAL (PURITY): Thiamethoxam (98.8% a.i.)

SYNONYMS: CGA 293343

CITATION: Brammer, A. (2003) Thiamethoxam: Preliminary Developmental Neurotoxicity Study in Rats. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Study number RR0935; May 22, 2003. MRID 46028201. Unpublished

EXECUTIVE SUMMARY: In a preliminary developmental neurotoxicity study (MRID 46028201), Thiamethoxam (98.8% a.i., batch # P 506006) was administered to groups of 10 time-mated female Alpk:AP$_f$SD (Wistar-derived) in the diet at dose levels of 0, 1000, 2500 or 5000 ppm from gestation day 7 *through post partum* day 22 *(inclusive)*. Mean intake during gestation was 0, 92.3, 212.5 and 362.1 mg/kg/day at 0, 1000, 2500 and 5000 ppm, respectively. The mean intake during lactation was 0, 156.5, 395.8 and 740.6 at 0, 1000, 2500 and 5000 ppm, respectively. Maternal clinical signs, body weight and food consumption (during gestation) were assessed. The number, survival, clinical signs and body weight of pups were evaluated.

There were no deaths or clinical signs of toxicity in maternal animals during gestation and lactation. Dams at 2500 and 5000 ppm had statistically significantly lower body weight (96-99% and 89-96% of control value, respectively) from days 8-22 of gestation. Body weight gain during gestation was decreased at 2500 ppm (88% of control value) and 5000 ppm (78% of control value). Food consumption was significantly decreased in dams at 2500 ppm (86-88% of control value) and 5000 ppm (65-74% of control value) for days 11-18 and 7-22 of gestation, respectively. During lactation, maternal body weight was significantly decreased (87-95% of control value) from days 1-22 at 5000 ppm. Food consumption during lactation was decreased in females at 2500 ppm (88-96% of control value; significant for days 8-11) and 5000 ppm (68-84% of control value; significant for days 8-22).

There were no treatment-related effects on reproductive parameters in dams or litter size, live born index, sex distribution or survival in pups. Body weight in male and female pups born to dams at 5000 ppm were significantly lower at birth (86% of control value) and remained lower (81-97% of control values, based on adjusted means) throughout lactation. Body weight was significantly lower (92% of control value) in males at 2500 ppm at birth but was comparable to

controls throughout lactation, based on adjusted means. Body weight gain was significantly decreased in males (72-77% of control value) and females (73-77% of control value) at 5000 ppm throughout lactation, in males at 2500 ppm (89-91% of control value) on days 15 and 22 and non-significantly in females at 2500 ppm (94-95% of control value) on days 15 and 22.

The maternal LOAEL was 2500 ppm based on decreased body weight gain and food consumption. The maternal NOAEL was 1000 ppm.

The offspring LOAEL was 2500 ppm based on decreased body weight gain. The offspring NOAEL was 1000 ppm.

This preliminary developmental neurotoxicity study in the rat is **Acceptable** (Non-guideline); it was conducted to determine dose levels for the definitive study.